

CLAIMS

What is claimed is:

- 5 1. A method for detecting the presence of a difference between two related polynucleotide sequences said method comprising:
 - a. forming a four-way complex comprising both of said polynucleotide sequences in duplex form;
 - 10 b. subjecting said four-way complex to branch migration conditions wherein, if a difference between said two related nucleic acid sequences is present, branch migration in said four-way complex ceases and said four-way complex is stabilized, and wherein, if no difference between said two related nucleic acid sequences is present, branch migration in said four-way complex continues until complete strand exchange occurs and said four-way complex resolves into two duplex nucleic acids, thereby forming a stabilized four-way
15 complex;
 - c. subjecting said stabilized complex or its resolved duplex products after branch migration to conditions allowing the specific binding of a first reagent that selectively recognizes a four-way complex, wherein the binding of said reagent to said four-way complex produces a detectable signal; and
 - 20 d. detecting the signal produced upon the specific binding of said first reagent to said four-way complex as indicative of the presence of said four-way complex, the signal thereof being related to the presence of said difference between said nucleic acid sequences and the failure to detect said signal thereof being related to the lack of difference between said nucleic acid sequences.
- 25 2. The method of claim 1 wherein said difference is a mutation.
3. The method of claim 1 wherein said nucleic acid sequences are DNA.
- 30 4. The method of claim 1 wherein said four-way complex comprises a Holliday junction.
5. The method of claim 1, wherein said first reagent is a chemical that binds to a Holliday junction and produces a specific, detectable signal upon binding to a Holliday
35 junction.

6. The method of claim 5, wherein said first reagent is a dye.
7. The method of claim 1 wherein said first reagent is a Holliday junction-binding protein.
- 5 8. The method of claim 7, wherein said Holliday junction-binding protein is a recombinase or a resolvase.
9. The method of claim 7 wherein said Holliday junction-binding protein is
- 10 thermostable.
10. The method of claim 7, where said Holliday junction-binding protein is selected from the group consisting of RuvA, RuvC, RuvB, RusA, RuvG, Cce1 and spCce1, Hjc.
- 15 11. The method of claim 5, wherein production of said detectable signal involves a conformational change in said Holliday junction-binding protein.
12. The method of claim 1, wherein production of said detectable signal involves
- 20 a Holliday junction induced association between said Holliday junction specifically binding protein(s) and said nucleic acids forming said Holliday junction complex.
13. The method of claim 1, wherein production of said detectable signal involves a specific Holliday-junction-binding-induced fluorescence of said first reagent.
- 25 14. The method of claim 1, wherein at least one of the related polynucleotide sequences is not detectably labeled.
15. The method of claim 14, wherein neither of the related polynucleotide
- 30 sequences is detectably labeled.
16. The method of claim 1, wherein:
- a. said stabilized complex is subjected to conditions allowing the specific binding of a second reagent that selectively recognizes a four-way complex,

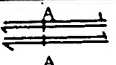
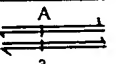
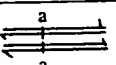
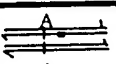
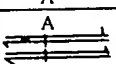
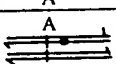
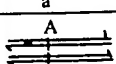
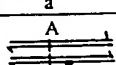
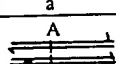
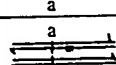
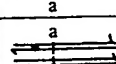
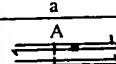
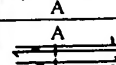
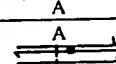
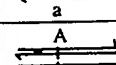
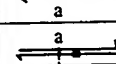
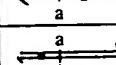
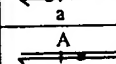
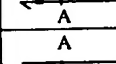
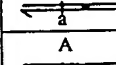
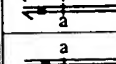
wherein the concurrent binding of said first and second reagents to said four-way complex produces a detectable signal; and

- 5 b. the signal produced upon the specific binding of said first and second reagents to said four-way complex is detected as indicative of the presence of said four-way complex, the signal thereof being related to the presence of said difference between said nucleic acid sequences and the failure to detect said signal thereof being related to the lack of difference between said nucleic acid sequences.

- 10 17. The method of claim 9, wherein said first and second reagents are Holliday-junction binding proteins.

18. The method of claim 9, wherein said signal is produced by Holliday junction-induced close association between said first and second reagents.

Table 1 Determining the genotypes of diploid genomic DNA samples using PCR

Target Genomic DNA sample (X/X)	Reference DNA (A/A)		Reference DNA (a/a)		Bar code for each genotype
	L(X/X)/(A/A) Mixture	R(X/X)/(A/A) Mixture	L(X/X)/(a/a) Mixture	R(X/X)/(a/a) Mixture	
 (A/A)	-/-	-/-	+/+	+/+	0022
 (A/a)	-/+	-/+	+/-	+/-	1111
 (a/a)	+/+	+/+	-/-	-/-	2200
 (A/A')	-/-	+/-	+/+	+/+	0122
 (A'/A)	+/-	-/-	+/+	+/+	1022
 (A'/a)	-/+	+/+	+/-	+/-	1211
 (A/a')	+/+	-/+	-/-	+/-	2111
 (A/a')	-/+	-/+	+/-	+/+	1122
 (A'/a')	-/+	-/+	+/+	-/+	1121
 (a/a')	+/+	+/+	-/-	+/-	2201
 (a'/a)	+/+	+/+	+/-	-/-	2210
 (A'/A')	+/+	+/+	+/+	-/-	0222
 (A'/A')	+/+	-/-	-/-	+/+	2022
 (A'/a')	-/-	-/-	+/+	+/+	1212
 (A'/a')	-/-	-/-	+/+	+/+	2121
 (a'/a')	+/+	+/+	-/-	+/+	2202
 (a'/a')	+/+	+/+	+/+	-/-	2220
 (A'/A')	-/+	+/-	+/+	+/+	1122
 (A'/a')	+/+	-/+	+/-	+/+	2112
 (A'/a')	-/+	+/+	+/+	+/-	1221
 (a'/a')	+/+	+/+	+/-	-/+	2211